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14. ABSTRACT
Hypothesis: a multi-modal optical spectroscopic method and an integrated needle probe can be developed for guiding needle biopsy for prostate cancer diagnosis. Multi-modal optical measurements to be utilized for the study are (1) light scattering spectroscopy (LSS), (2) auto-fluorescence spectroscopy (AFS), and (2) auto-fluorescence life-time measurements (AFLT). Our specific aims are:

Aim 1: to develop a multi-modal, optical spectroscopic instrument, which allows the measurements of (1) LSS, (2) AFS, and (3) AFLT. The proposed system will be portable, can be used for in vivo measurements, and collect and present the data in real time. Aim 2: to integrate the optical fibers, which collect light scattering and auto-fluorescence from the prostate tissue, into a transrectal-ultrasound, needle-biopsy probe. In the development phase, the optical signatures of prostate cancer can be collected with the biopsy tissues and identified along every tract of needle biopsies. Aim 3: to collect optical signals of control and cancer tissues ex vivo along with the regular human needle biopsy, followed by classification algorithm development to discriminate cancer tissues. Aim 4: to perform in vivo measurement from human subjects to obtain the accuracy and sensitivity of the integrated probe in order to provide real-time, on-site, improved guidance for prostate cancer tissue biopsy.

15. SUBJECT TERMS
Technology Development, optical spectroscopy, transrectal probe, optical biopsy, auto-fluorescence spectroscopy, fluorescence life-time measurement

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2010-2011 Annual Progress Report

This report presents the specific aims and accomplishments of our prostate cancer research project during the year of funding sponsored by the US Department of the Army. It covers our activities from September 1, 2010 to December 31, 2011.

1. Introduction

The overall hypothesis for this study is that a multi-modal optical spectroscopic method and an integrated needle probe can be developed for guiding needle biopsy for prostate cancer diagnosis. Multi-modal optical measurements to be utilized for the study were (1) light reflectance spectroscopy (LRS), (2) auto-fluorescence spectroscopy (AFS), and (3) auto-fluorescence life-time measurements (AFLM).

The project has four specific aims:

Aim 1: to develop a multi-modal, optical spectroscopic instrument, which allows the measurements of (1) LRS, (2) AFS, and (3) AFLM. The proposed system will be portable, can be used for in vivo measurements, and collect and present the data in real time.

Aim 2: to integrate the optical fibers, which collect light scattering and auto-fluorescence from the prostate tissue, into a transrectal-ultrasound, needle-biopsy probe. In the development phase, the optical signatures of prostate cancer can be collected with the biopsy tissues and identified along every tract of needle biopsies.

Aim 3: to collect optical signals of control and cancer tissues ex vivo, followed by classification algorithm development to discriminate cancer tissues.

Aim 4: to perform in vivo measurement from human subjects to obtain the accuracy and sensitivity of the integrated probe in order to provide real-time, on-site, improved guidance for prostate cancer tissue biopsy.

2. Body of the Report

We completed Aim 1 in our Year 1 effort and reported it in Year 1 report. This report summarizes the work that we performed in Year 2 from Sept. 1, 2010 to Dec. 31, 2011, mainly for Aims 2 and 3. We will also provide the information on the corresponding achievements obtained during this period of time, as given below.

2.1 Report for Aim 2

Aim 2: *to integrate the optical fibers, which collect light scattering and auto-fluorescence from the prostate tissue, into a transrectal-ultrasound, needle-biopsy probe. In the development phase, documentation to obtain IRB approval for ex vivo human prostate measurements needs to be prepared and obtained.*

2.1.1 Assembly of the integrated LRS/AFLM system

In Year 1, we assembled two separate units for LRS and AFLM and tested them using laboratory tissue phantoms. In Year 2, we have completed and integrated them together as a combined system [see Fig. 1(a)] by having an integrated fiber optic probe, as shown in Fig. 1(c) below. The outer diameter of the probe tip is 1 mm, while the inner fiber diameters vary between 100 μm to 400 μm. Specifically, the two 200 μm are connected to the LRS unit for light reflectance measurements, while the 100 μm is to deliver the excitation light at the chosen wavelength (447 nm) and 400 μm is to collect the auto fluorescence light. In this way, the two modalities (LRS and AFLM) can be used sequentially without

moving the fiber probe, essentially measuring the same area of tissue. Respective light sources are manually turned on before each acquisition and turned off after. Overall, the dual-modality data acquisition consists of 4 lifetime measurements at four emission wavelengths (532 nm, 562 nm, 632 nm, 684 nm) and 1 reflectance spectrum. Data processing and analysis will be performed offline.

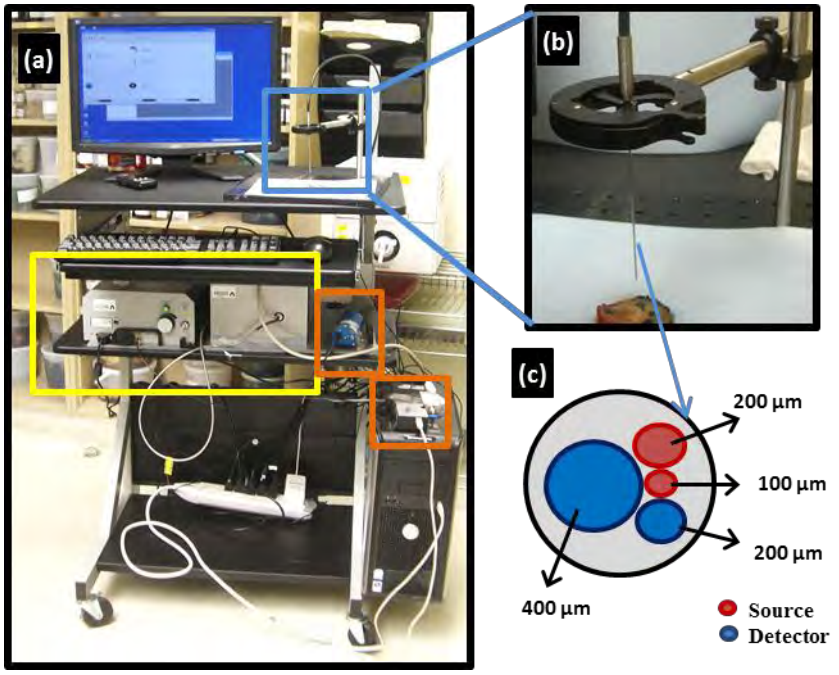

Fig. 1 (a) A photograph of the combined LRS/AFLM system for *ex vivo* human prostate specimen measurements. The yellow square contains the main AFLM hardware. The orange squares contain LRS hardware, namely the broadband light source and a CCD array spectrometer. (b) An inset of (a) showing a close up of the measurement geometry with the integrated probe, probe holder and tissue sample. (c) Detailed geometry of the probe tip. Two 200-µm fibers are connected to the LRS system; the other two fibers go to the AFLM system.

2.1.2 Protocol development for *ex vivo* data acquisition from human prostate glands

After successful implementation of an integrated LRS/AFLM system, an *ex vivo* protocol has been studied and designed to evaluate the signatures of human prostate cancer. Study population will be selected based on the biopsy records of the patients. Selection criteria target on high grade prostate cancer (i.e., Gleason score ≥ 7) with large cancer volumes (assessed by % involvement of the biopsy cores). Fresh prostatectomy sample will be brought to the pathology room immersed in saline, within a few minutes after it is extracted from the patient. Following are the planned sequence of steps involved in measurement protocol, as illustrated in Fig. 2:

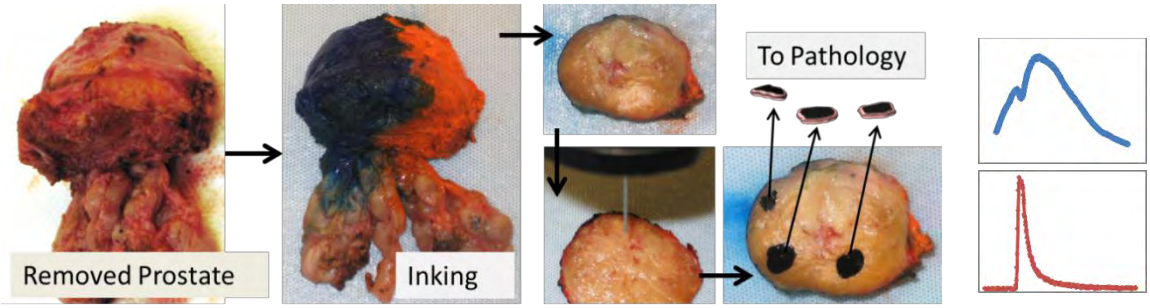

Fig. 2 A schematic flow diagram describing *ex vivo* human study protocol: the removed prostate is first inked and then bivalved. Optical readings will be taken on 3 identified regions for cancer, normal and BPH. The measured tissues will be then sliced out and sent to pathology for analysis. Blue and red curves on the right are representative measurements for LRS and AFLM, respectively.

Specifically, the planned protocol for *ex vivo* data acquisition from human prostate glands is given as follows:

(1) Pathologist notes the physical measures of the excised prostate, and inks the prostate surface as per the regular pathology protocol (blue dye for left side, orange dye for right side, green dye for anterior margin).
(2) Prostate is profusely washed with acetic acid multiple times to fix the dyes (so that dyes do not penetrate the tissue and contaminate it for optical measurement).
(3) Based on the patent's previous biopsy record and palpation of the prostate, it is bivalved at an optimal location to expose the cancer nodule/lesion. If it is unclear to see cancer lesions, a quick-prep procedure is performed to identify the cancer region.
(4) On the exposed inner surfaces, three main regions are identified visually: cancer, normal, and benign prostate hyperplasic (BPH)/ transition zone tissue.
(5) The integrated fiber probe tip is placed on the tissue surface [see Fig. 1(b)], and the readings are taken sequentially using LRS and AFLM. Eight random points will be measured in each selected sub-regions. In most cases, therefore, 24 locations (8 from each tissue type) are measured using this combined LRS/AFLM system.
(6) After measurement, the three measured regions are marked with black dye, and sliced (~ 1 mm thick) and stored in formalin after loading in separate cassettes with labels.
(7) Each region is then analyzed by the pathologist and used as "gold standard" to categorize the optical measurements for comparison and further analysis.

2.1.3 Preparation of documentation to obtain IRB approval for *ex vivo* human prostate measurements

Based on the two tasks completed in Sections 2.1.1 and 2.1.2, we prepared and submitted the needed documentation to both the University of Texas Southwestern Medical Center at Dallas and the University of Texas at Arlington in order to obtain IRB approval for *ex vivo* human prostate measurements. By the end of this reporting period, we just obtained the IRB approval for the study.

2.2 Report for Aim 3

Aim 3: *to collect optical signals of control and cancer tissues ex vivo, followed by classification algorithm development to discriminate cancer tissues.*

2.2.1 Preliminary AFLM Data:

The AFLM output has a shape of exponential decay, so we express the fluorescence decay using a double exponent model with a baseline correction, as written in eq. (1), to fit the measured lifetime data. In eq. (1), *I(t)* represents the normalized fluorescence intensity; a_1, a_2, τ_1 and τ_2 are the fitted parameters and represent amplitude and lifetime of individual decay components in the two component model. Intensity weighted mean-lifetime (τ_m) can also be calculated using eq. (2).

$$I(t) = a_1 e^{t/\tau 1} + a_2 e^{t/\tau 2} + c \qquad (1)$$

$$\tau_m = \frac{a_1\tau_1^2 + a_2\tau_2^2}{a_1\tau_1 + a_2\tau_2} \qquad (2)$$

Since AFLM curve fitting yields 5 parameters (a_1, a_2, τ_1, τ_2, and τ_m) for each lifetime curve and we utilize 4 emission filters in the measurement, we can obtain 20 parameters (4 wavelengths X 5 fitted parameters) for each measured point on the tissue. In our very preliminary data collection and analysis within this report period, we have collected *ex vivo* data from several human prostate specimens, but are not yet able to analyze these parameters to examine possible differences between cancer and normal prostate tissues, as well as the differences between cancer and BPH tissues. We are continuing to collect the human specimen data and perform more comprehensive data analysis.

2.2.2 Preliminary LRS Data:

LRS data was analyzed for 4 cases, all of them being Gleason score 9 grade. All the reflectance spectra were calibrated with a standard reflectance sample. The averaged reflectance spectrum for cancer and normal tissue is shown in Fig. 3. Next, the absolute values of [HbO], [Hb], and μ_s' were calculated using the method developed in *Aim 1*, as reported in Year 1report.

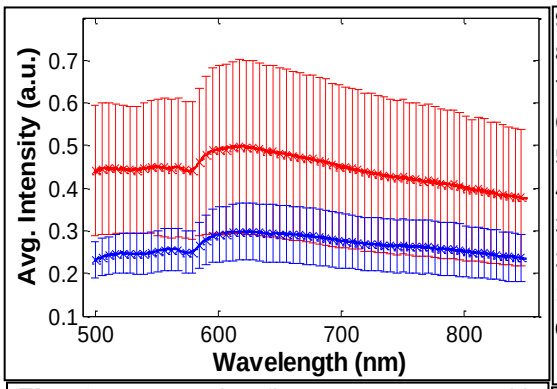

Fig. 3 Averaged reflectance spectra with standard deviation for cancer (red) and normal *(blue) tissue.*

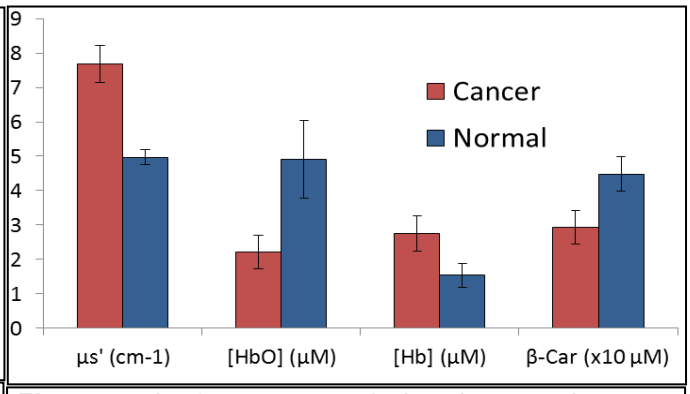

Fig.4 Results from LRS analysis. The error bars are standard errors. N=40 for cancer; 36 for normal tissue. From left to right, p-value =$3x10^{-5}$, 0.03, 0.05, 0.03.

Figure 4 shows the calculated absolute values of four LRS parameters, oxy-hemoglobin concentration, HbO, deoxy-hemoglobin concentration, Hb, light scattering coefficient, $\boldsymbol{\mu_s'}$, and concentration of β-carotene. It is found that $\boldsymbol{\mu_s'}$ was significantly larger for cancer, indicating higher scattering in cancerous tissue. Also, [HbO] was reduced and [Hb] elevated in cancerous regions, indicating higher oxygen consumption by cancer cells. However, it can be seen that the [Hb] and [HbO] values are very small, indicating low levels of blood in the *ex vivo* samples. Here, β-carotene was also included in the comparison in addition to [HbO] and [Hb] as an absorber. It was found to be reduced in cancer as compared to normal tissues. The data obtained so far are very promising to differentiate cancer from normal tissue, with p-values of $3x10^{-5}$, 0.03, 0.05, and 0.03 for , $\boldsymbol{\mu_s'}$, HbO, Hb, and β-carotene. We are continuing to collect the human specimen data and perform more comprehensive data analysis for LRS portion too.

3. Key Research Accomplishments and Reportable Outcomes

(1) We have integrated LRS and AFLM into one combined system with a unified fiber optic probe, which can collect light scattering reflectance and auto-fluorescence signals from the same region of *ex vivo* prostate tissues without moving the probe;
(2) We have obtained the final approval of IRB for taking *ex vivo* human prostate measurements;
(3) We have collected optical signals from control and cancer tissues *ex vivo* and showed promises to be able to differentiate cancer from normal prostate tissues, based on LRS.
(4) Several related research works are published:
  (a) Sangram Raut, Amber Heck, Jamboor Vishwanatha, Pabak Sarkar, Avani Mody, Rafal Luchowski, Zygmunt Gryczynski, Ignacy Gryczynski, "Fluorescent properties of antioxidant cysteine ABZ analogue," Journal of Photochemistry and Photobiology B: Biology 102 (2011) 241–245.
  (b) Rafal Fudala, Amalendu P. Ranjan, Anindita Mukerjee, Jamboor K. Vishwanatha, Zygmunt Gryczynski, Julian Borejdo, Pabak Sarkar and Ignacy Gryczynski, "Fluorescence Detection of MMP-9. I. MMP-9 Selectively Cleaves Lys-Gly-Pro-Arg-Ser-Leu-Ser-Gly-Lys Peptide," *Current Pharmaceutical Biotechnology,* 2011*, 12*, 834-838

4. Conclusions and plan for next year

In summary, for the report period from Sept. 2010 to December 2011, we have implemented an integrated unit for AFLM and LRS and demonstrated that both AFLM and LRS are feasible methods to collect good optical signals from *ex vivo* human prostate tissue specimens. Further measurements from a larger sample size of human prostate specimens will be explored in the following year.

Specifically, in the coming year, we will carry on the development for Aims 3 and 4, as listed below:

1) Both AFLM and LRS data will be further measured and analyzed;
2) There are multiple approaches to feature selections and cancer classification. Data classification will be implemented using at least two methods;
3) Both AFLM and LRS data will be analyzed separately for all the subjects, and classification algorithms will be implemented to see the performance of each of the two modalities for cancer, normal and BPH tissue types;
4) Feature selections will be performed across the two modalities, and the performance of the combined technique will be compared against each individual technique.
5) ROC (receiver operating characteristic) curve will be plotted using the entire data set for training the model, and entire dataset for testing the model. Also, we will use a k-fold cross-validation technique for more accurate results.
6) Manuscripts will be prepared and submitted.